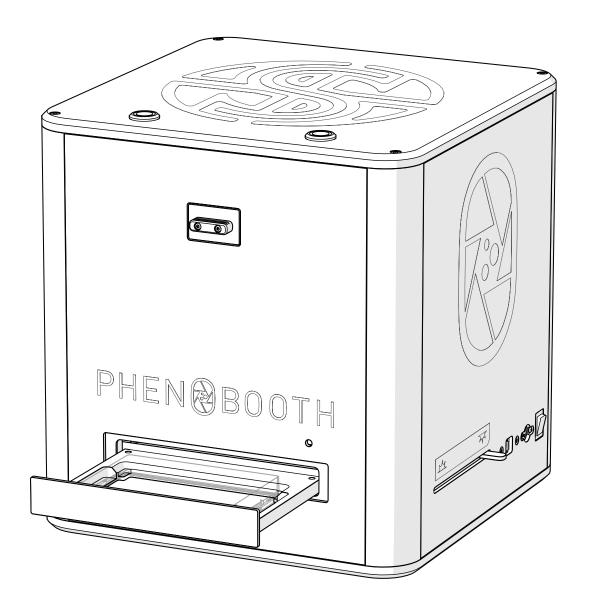
PHEN@BOOTH™





\triangle

READ BEFORE YOU START!

- · Remove plate lids before imaging. If lids are in place when the input tray is closed, the machine will crash and may be damaged.
- · Keep your hands clear while the input tray is moving. Catching your hands in the input tray may cause injury.
- Ensure power supply is the correct way round. Plugging the power supply into the PhenoBooth upsidedown will cause damage to the machine. See p.10 for details.
- Avoid plugging the USB 3.0 cable in slowly. A common error with all USB 3.0 devices is that they are recognised as USB 2.0. This happens when the USB plug is inserted too slowly.
- Remove plate and petri adaptor before moving PhenoBooth. If you need to move the PhenoBooth for any reason, make sure any plates are removed from the machine as well as the petri dish adaptor.
 These items can fall from the input tray and become lodged inside the PhenoBooth.

- 2. Before you start!
- 3. Introduction
- 5. OUT OF THE BOX
- 6. Anatomy & features
- 9. Unboxing & installation
- 12. LET'S START!
- 13. PhenoBooth operation
- 14. COLONY COUNTING PROJECT
- 15. New project
- 16. Image acquisition
- 18. Pre-processing
- 19. Detection
- 20. Filtering
- 21. Export
- 22. COLONY SCREENING PROJECT
- 23. New project
- 24. Image acquisition
- 26. Pre-processing
- 27. Detection
- 28. Filtering
- 29. Analysis
- 30. Visualisation
- 31. Export

- 32. RCP PROJECT
- 33. New project
- 34. Image acquisition
- 36. Pre-processing & detection
- 37. Filtering
- 38. Stinger Export
- 39. ROTOR HDA / The Stinger
- 3. BEST PRACTICE
- 44. Software icons
- 46. Feedback light
- 47. Batch processing
- 48. History plug-in
- 49. Add new plate run
- 50. Import/export Project
- 52. Using filters
- 53. Notes

PHENOBOOTH™

INTRODUCTION

The PhenoBooth is a fast, semi-automated plate reader for imaging and analysis of cell colonies. The PhenoBooth can image standard SBS plates, Petri dishes and Singer PlusPlates.

USER GUIDE

Follow these instructions to get the most out of the PhenoBooth. This guide outlines getting started and general operation, as well as software tutorials for each package.

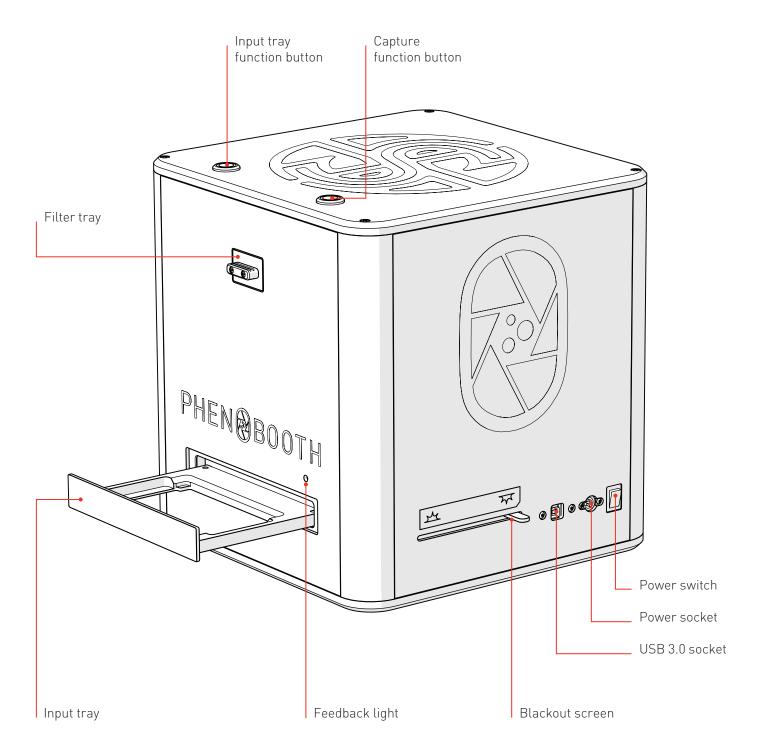
DISCLAIMER

At Singer Instruments, we are constantly seeking to improve our products and adapt them to the requirements of modern research techniques and testing methods. This involves modification to the mechanical structure and optical design of our instruments. Therefore, all descriptions and illustrations in this user guide, including all specifications are subject to change without notice. To download the latest version of this user guide please visit: <code>singerinstruments.zendesk.com</code>

OUT OF THE BOX

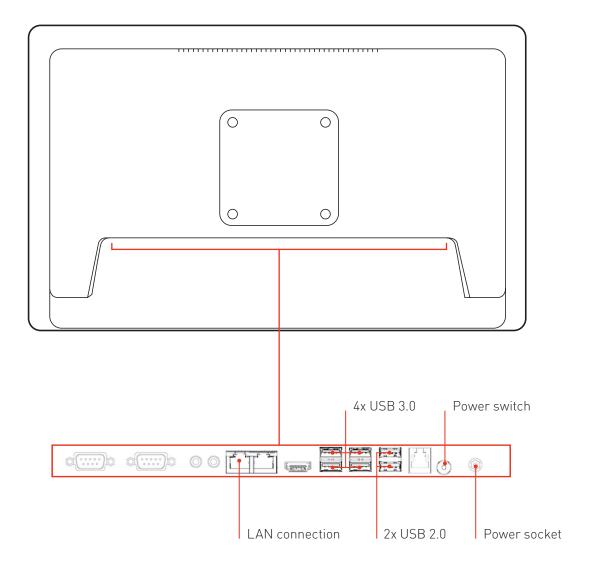
Find out what comes with the PhenoBooth. We'll take you through the steps involved in unboxing and assembling ready for the exciting world of colony imaging!

PHENOBOOTH



ANATOMY & FEATURES

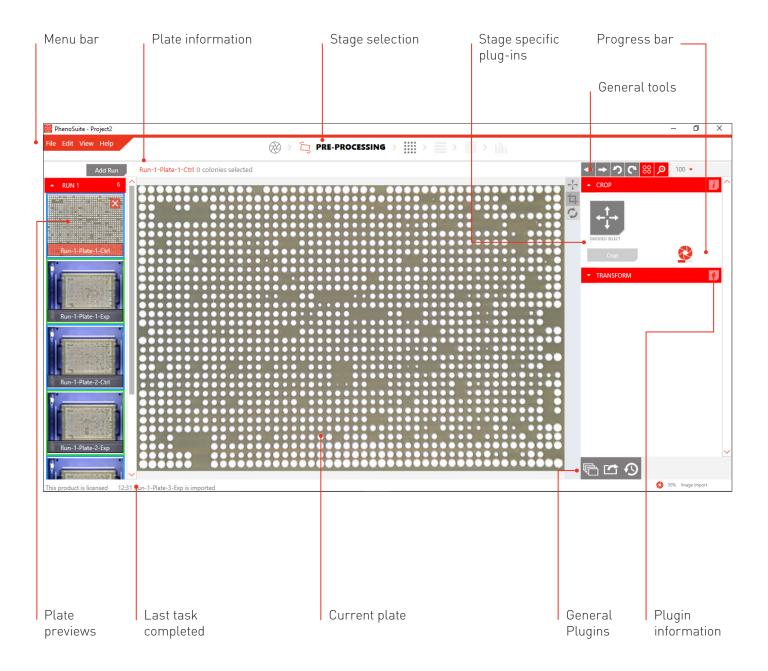
MCI SCREEN (BACK)



singerinstruments.com

ANATOMY & FEATURES

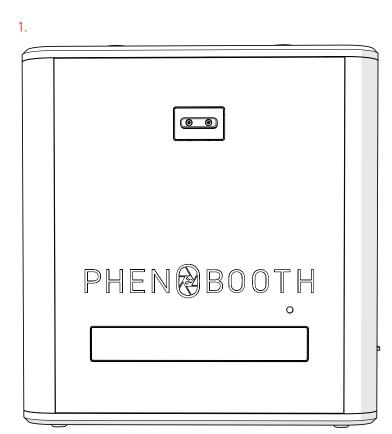
SOFTWARE

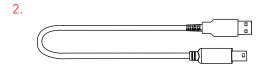


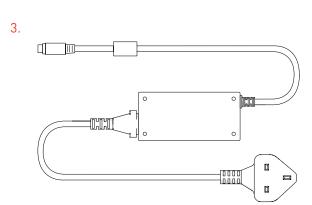
UNBOXING & INSTALLATION

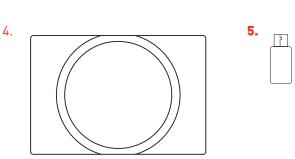
IN THE PHENOBOOTH BOX

- 1. PhenoBooth
- 2. USB 3.0 cable
- 3. Power supply
- 4. 90mm petri adapter (optional extra)
- 5. Software USB stick



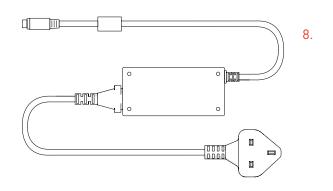


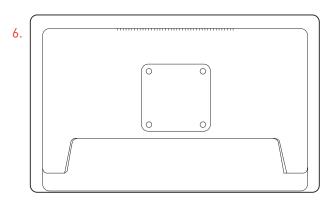




IN THE MCI BOX

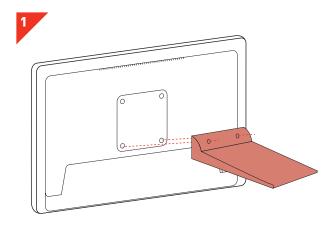
- 6. MCI Screen
- 7. MCI Stand & screws
- 8. MCI Power Supply
- 9. Mouse (coming soon)
- 10. Keyboard (coming soon)



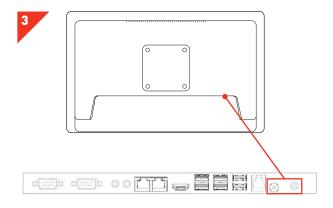




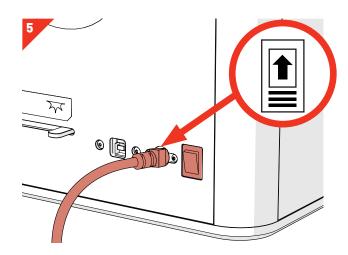
UNBOXING & INSTALLATION



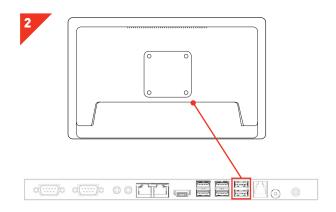
· Carefully remove the MCI screen from the box and attach stand using the screws provided..



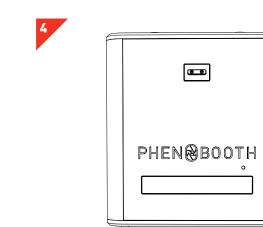
· Connect the MCI power cable to the power socket and turn on the power switch.



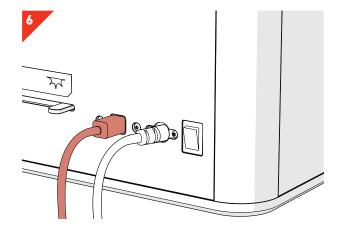
- · Insert PhenoBooth power supply and turn on the power switch.
- · WARNING: Make sure the arrow diagram is on top of the cable. Inserting the power supply upsidedown will cause damage to the PhenoBooth.



· Connect the mouse and keyboard to the MCI screen via the USB 2.0 sockets.

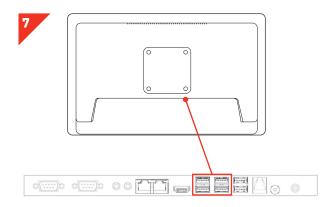


- · Carefully remove the PhenoBooth from the box and stand on your workspace.
- HEAVY: May require 2 people.

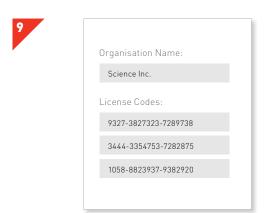


• Insert the USB cable into the PhenoBooth USB 3.0 socket and to the MCI USB 3.0 socket.

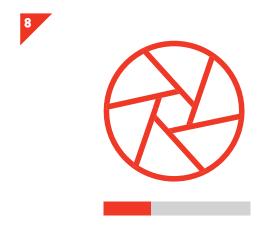
UNBOXING & INSTALLATION



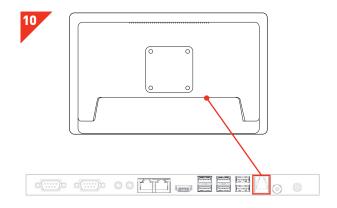
· Insert the USB stick into the MCI via a USB 3.0 socket.



 After the software and dependencies are installed, enter the organisation name and licence code that came with your PhenoBooth packing documents and click Activate



· Click on the PhenoBooth software setup icon to install software.



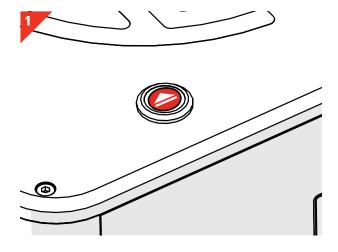
· For future software updates you will need to connect the MCI to the internet via the LAN socket.

LET'S START!

I'm ready to start!

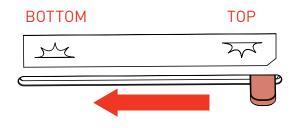
Here is a quick run-through of how to use the PhenoBooth and get started with the software — it's all very simple!

PHENOBOOTH OPERATION

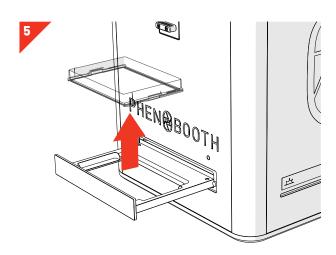


· Press the input tray function button to open the input tray.

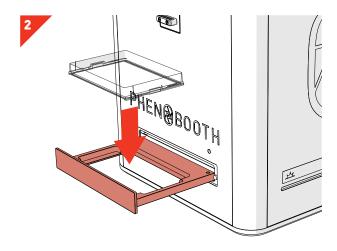




· Choose between top or bottom lighting by sliding the blackout screen (left for bottom and right for top).

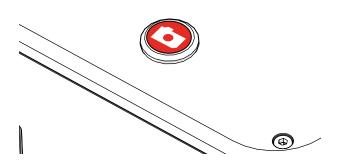


- · Press the input tray function button to open the input tray, remove your plate and replace the lid.
- · Repeat these steps for the rest of your plates.

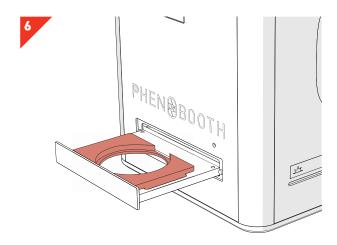


- · Remove the lid from your plate and place the plate into the input tray.
- WARNING: Ensure plate is nestled correctly to avoid a hardware crash.





- · Press the capture function button to image the plate.
- · Follow the software instructions.



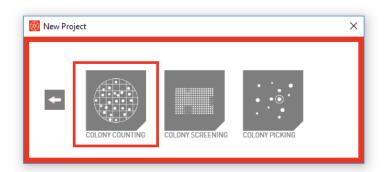
- · If using petri dishes, you will need to use a petri dish adaptor. Place the adaptor into the input tray.
- · Remove the petri dish lid and insert into the adaptor.

singerinstruments.com

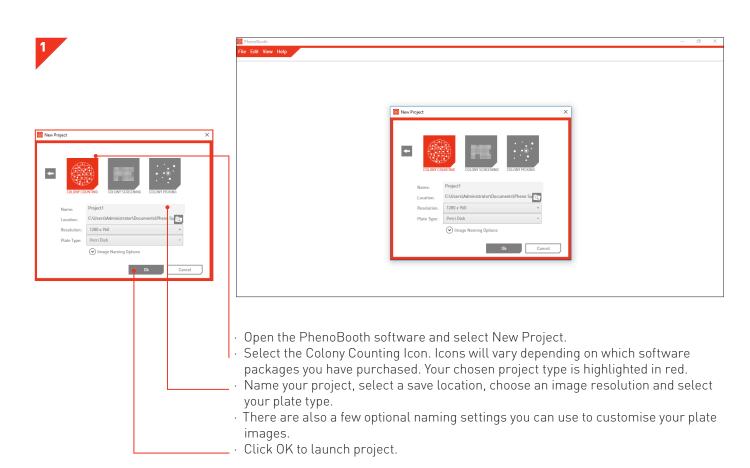


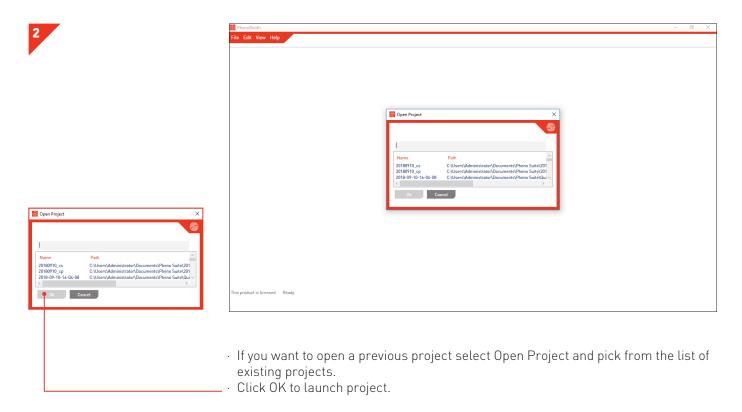
COLONY COUNTING PROJECT

Colony Counting is the basic package included with your PhenoBooth. There are 5 stages in the Colony Counting package: Image Acquisition, Pre-processing, Detection, Filtering and Summary.



COLONY COUNTING - NEW PROJECT

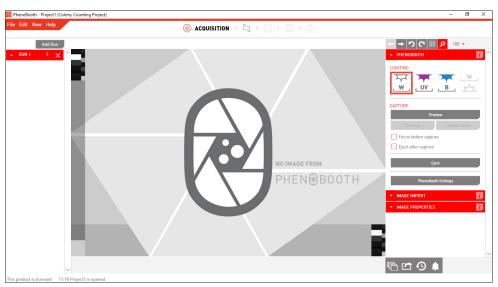




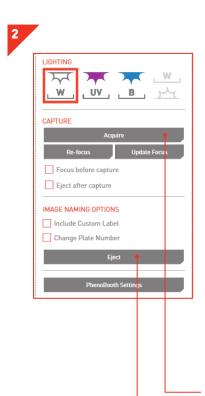
COLONY COUNTING - IMAGE ACQUISITION







- There are two options for acquiring an image: PhenoBooth or import an existing image.
- $\cdot\,$ Select the desired method from the plug-in panel on the right of the screen.
- If using the PhenoBooth camera, you can preview the image by pressing Preview.



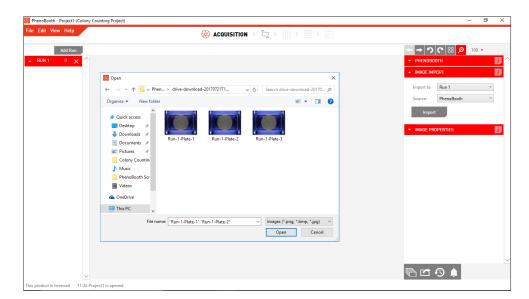


- · If using the PhenoBooth, click on that tab and select your desired light settings. Plate names are automatically generated.
- · Insert a plate into PhenoBooth and press Acquire.
- · Eject will allow you to remove the plate.
- · Repeated until all of your plates have been captured.
- moves onto the pre-processing stage.

COLONY COUNTING - IMAGE ACQUISITION



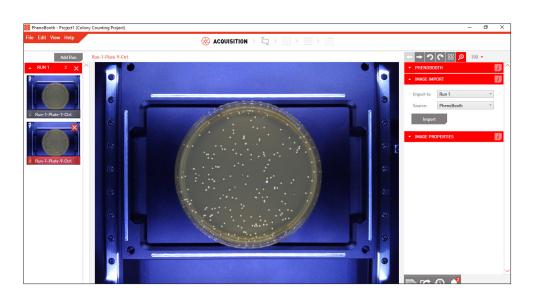




- If using existing images, click the Image Import tab. Here you can select which run the plate images will be associated. If this is your first run then Run 1 will be the only option. See p.49 to add new runs.
- · Click Import to select images from your computer.





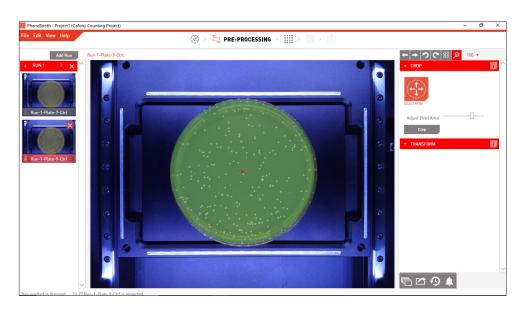


- · Your captured or imported images now appear on the left of the screen. The currently selected plate is highlighted with red.
- · moves onto the pre-processing stage.

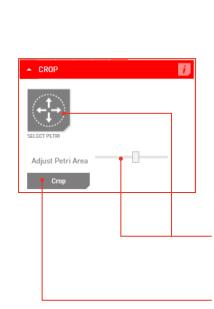
singerinstruments.com

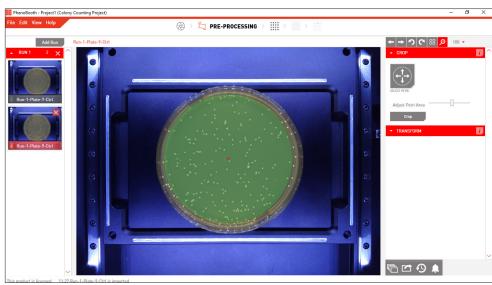
COLONY COUNTING - PRE-PROCESSING





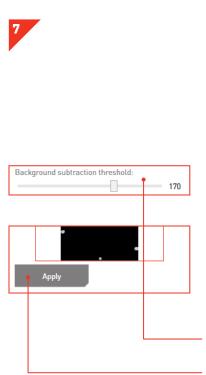
Select which image to process first. If using the batch process option, this plate's settings will be applied to the rest of the plates in the run.





- \cdot Click on Select Petri to automatically detect the plate area. You can adjust the plate area using the Petri Slider.
- $\cdot\,$ If using a PlusPlate, press the Select button and drag over the colony area of the plate.
- · Click Crop to remove the edge of the plate and beyond.
- moves to the Detection stage.

COLONY COUNTING - DETECTION



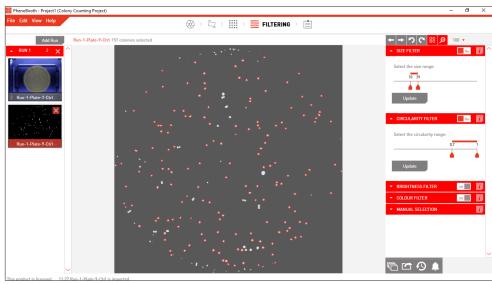


- \cdot In order to generate colony information, the background needs to be removed.
- · Use the slider to adjust the filter intensity if necessary.
- · Check that all the smaller colonies are visible in the preview window then click Apply.
- · moves to the Filtering stage.

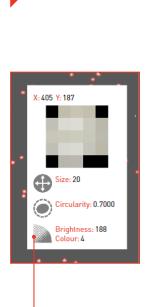
singerinstruments.com

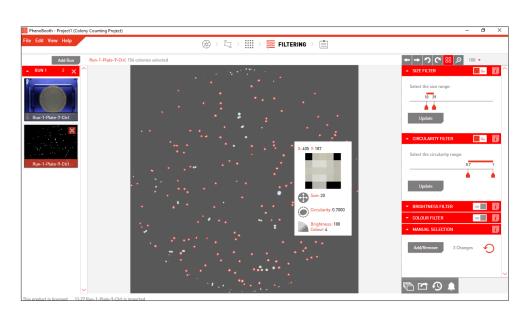
COLONY COUNTING - FILTERING





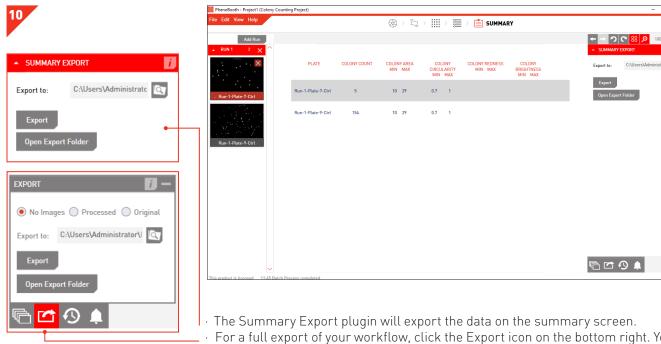
- Detected colonies are highlighted with a red circle. \bigotimes turns these highlights on or off.
- Use the filter options to set minimum and maximum values for size, circularity, brightness and colour for colony detection. You can use the size filter to discard items that are too large or small to be colonies. Click the Update button to apply.
- · Colonies can also be manually selected by clicking the Add/Remove button in the Manual Selection plug-in tab. Then simply click on a colony to select or deselect it.
- · You can batch process the rest of your plates with the same options using the instructions on p.47.





Hover over a colony to see it's size, circularity, brightness and colour properties in the Colony Info card.

COLONY COUNTING - SUMMARY



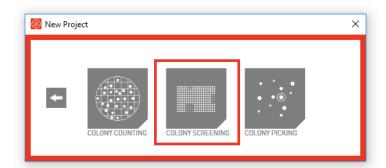
- · For a full export of your workflow, click the Export icon on the bottom right. You can choose to export no images, processed images or the original images.
- · The export includes: plate image (if selected), summary file and colony data table.
- The Summary File contains colony counts for all plates as well as colony imaging and filtering options information.
- The Colony Data Table contains position and properties of all detected colonies on all plates.



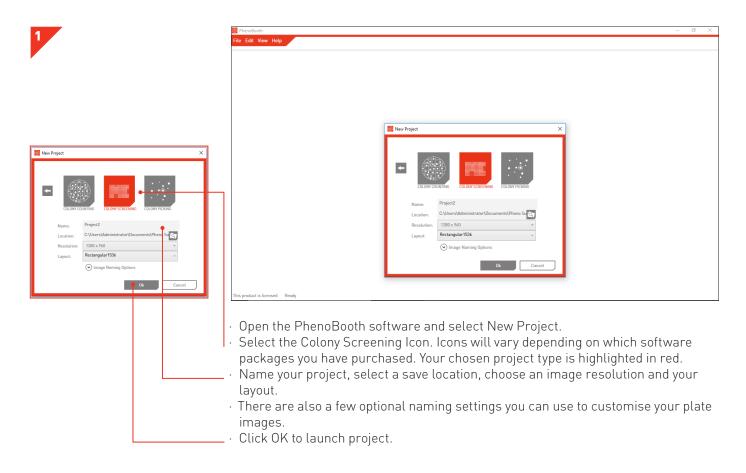
COLONY SCREENING PROJECT

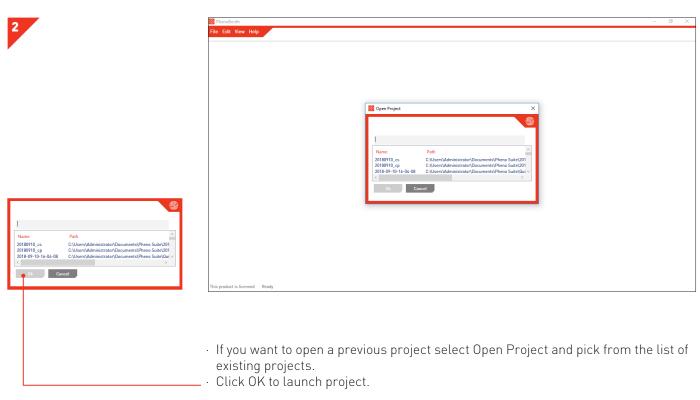
The Colony Screening package includes all the plug-ins you need for colony screening, such as size and circularity filters, plate normalisation, gene mapping, control / experiment comparison and heat map visualisation.

There are 6 stages in the Colony Screening package: Image Acquisition, Pre-processing, Detection, Filtering, Analysis and Visualisation.

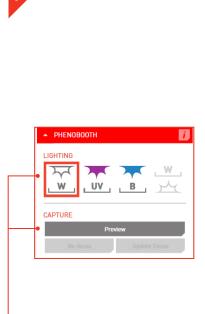


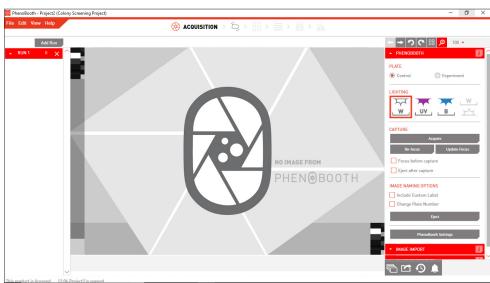
COLONY COUNTING - NEW PROJECT



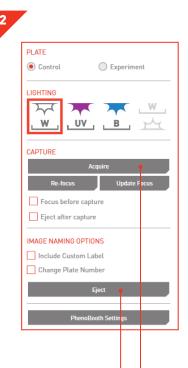


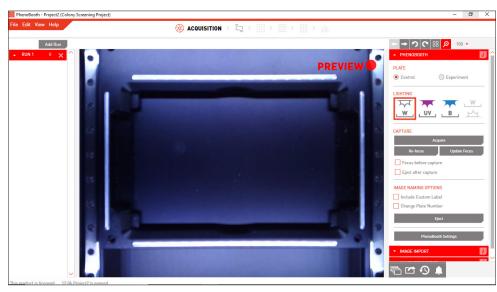
COLONY SCREENING - IMAGE ACQUISITION





- \cdot There are two options for acquiring an image: PhenoBooth or import an existing image.
- Select the desired method from the plug-in panel on the right of the screen. Press Preview to preview the image.



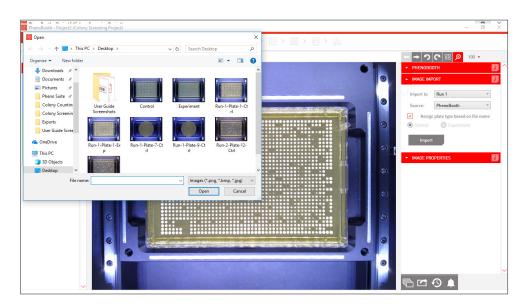


- If using the PhenoBooth, click on that tab and select your desired light settings and select either Control or Experiment. Plate names are automatically generated.
- · Insert a plate into PhenoBooth and press Acquire.
- · Eject will allow you to remove the plate.
- · Repeated until all of your plates have been captured.
- moves onto the pre-processing stage.

COLONY SCREENING - IMAGE ACQUISITION

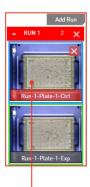


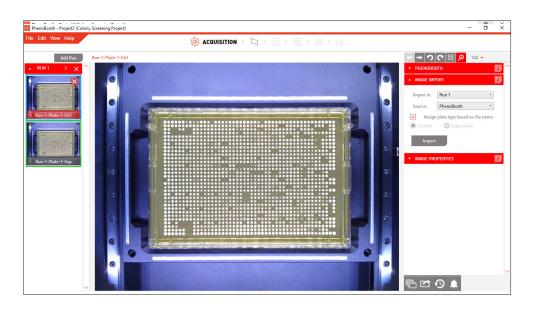




- · If using existing images, click the Image Import tab. Here you can select which run the plate images will be associated. If this is your first run then Run 1 will be the only option. See p.49 to add new runs.
- Select whether the image is a control or experiment, or allow the PhenoBooth software to assign based on name. For example: run-1-plate-1-ctrl or run-6-plate-9-exp.
- · Click Import to select images from your computer.





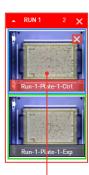


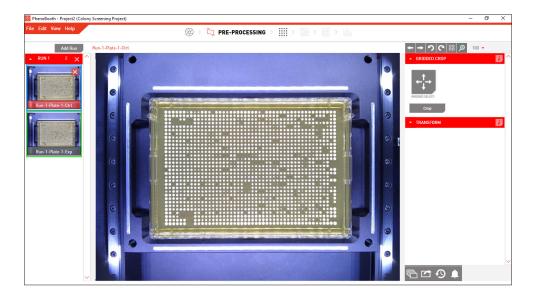
- · Your captured or imported images now appear on the left of the screen. The currently selected plate is highlighted with red. Control plates have a blue border and experiment plates have a green border.
- · moves onto the pre-processing stage.

singerinstruments.com

COLONY SCREENING - PRE-PROCESSING

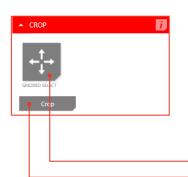






· Select which image to process first. If using the batch process option, this plate's settings will be applied to the rest of the plates in the run.

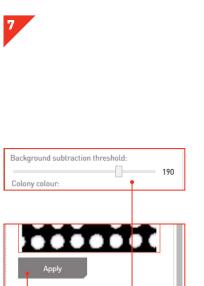


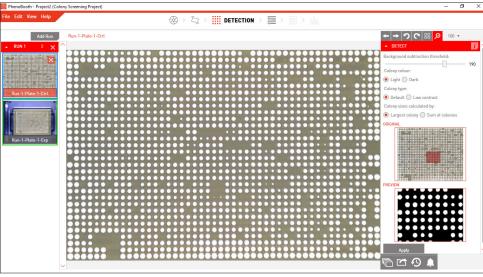




- · In the Crop plug-in select Gridded Select.
- The grid will automatically overlay the plate image. Press Crop to remove the edge of the plate and beyond.
- · moves to the Detection stage.

COLONY SCREENING - DETECTION





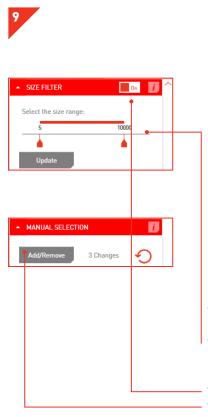
- · In order to generate colony information, the background needs to be removed.
- · Use the slider to adjust the filter intensity if necessary.
- · Check that all the smaller colonies are visible then click Apply.

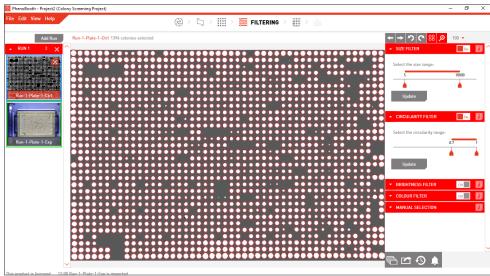




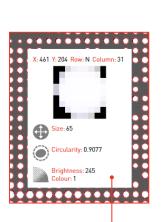
- The number of colonies and their array formation are shown above the plate image.
- moves to the Filtering stage. Here you can remove items such as debris on the plate or filter colonies by properties such as size or colour.

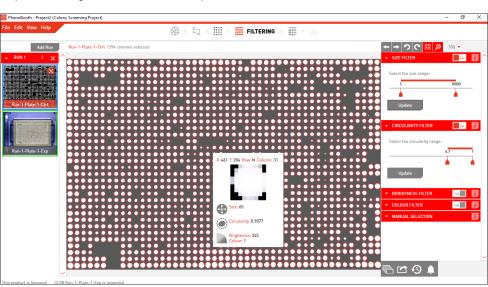
singerinstruments.com





- Detected colonies are highlighted with a red circle. \bigotimes turns these highlights on or off
- Use the filter options to set minimum and maximum values for size, circularity, brightness and colour for colony detection. You can use the size filter to discard items that are too large or small to be colonies. Click the Update button to apply.
- · Turn filters on and off using the toggle button.
- · Colonies can also be manually selected by clicking the Add/Remove button in the Manual Selection plug-in tab. Then simply click on a colony to select or deselect it.
- · Now is the best time to batch process the rest of your plates with the same options using the instructions on p.47.



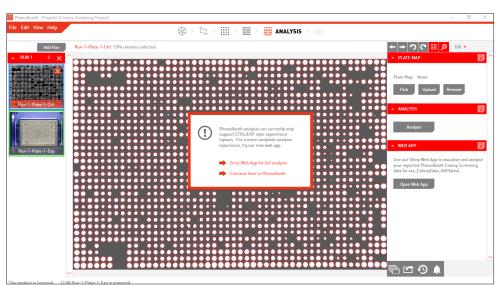


- · Hover over a colony to see it's size, circularity and colour properties in the Colony Info plug-in tab.
- . moves onto the Analysis stage.

COLONY SCREENING - ANALYSIS

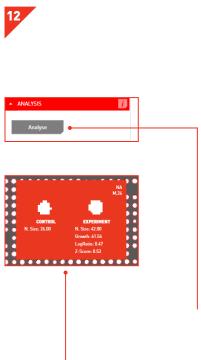


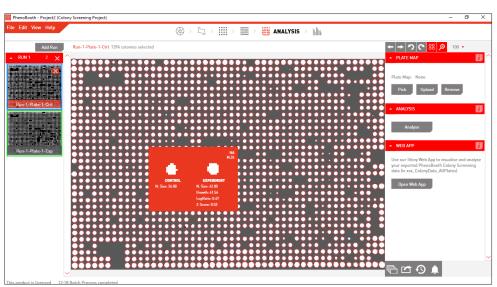




ADD TEXT HERE

- The PhenoBooth software will handle simple control/experiment comparison. For more thorough analysis we have an online app.
- · To move to the web app, select the Web App option from the pop-up window. You will be prompted to export your project at this stage.
- · Click the Continue line to stay in PhenoBooth.

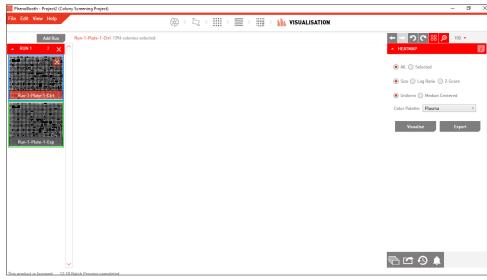




- To analyse colony growth difference between your current plate and it's control/experiment plate, select the Analysis tab and click Analyse.
- To compare control and experiment plates you must first process all plates. To do this use the batch process instructions on p.47.
- · When you place the cursor over a colony, the growth information for the control and experiment plate will be compared.
- · Colony co-ordinates and gene information (if available) are shown in the top right corner of the info box. You can upload or select a plate map using the Plate Map plugin.
- moves to the Visualisation stage.

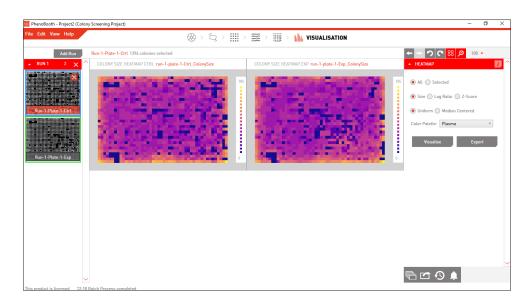
COLONY SCREENING - VISUALISATION





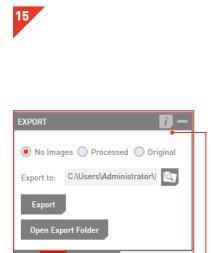
- · To view heat maps of Size, Log Ratio or Z-score, choose your visualisation options from the Heat Map tab, then click Visualise.
- \cdot You can select to visualise all of your plates or just a selection.

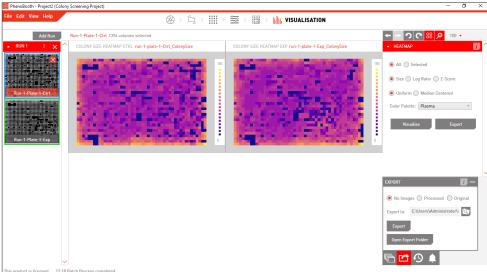




· Boom! You can now see heat maps for your selected plates.

COLONY SCREENING - EXPORT





- Export options are found in the Export tab. You can choose to export no images, processed images or the original images.
- · Image formats available are: .bmp, .jpeg, .png or .tiff.
- The export includes: plate image (if selected), summary file, colony data table and colony data/analysis screen.
- The Summary File contains colony counts for all plates as well as colony imaging and filtering options information.
- The Colony Data Table contains position and properties of all detected colonies on all plates.

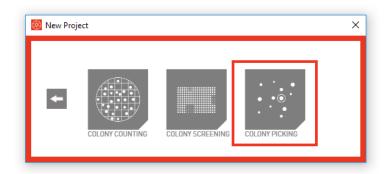
singerinstruments.com



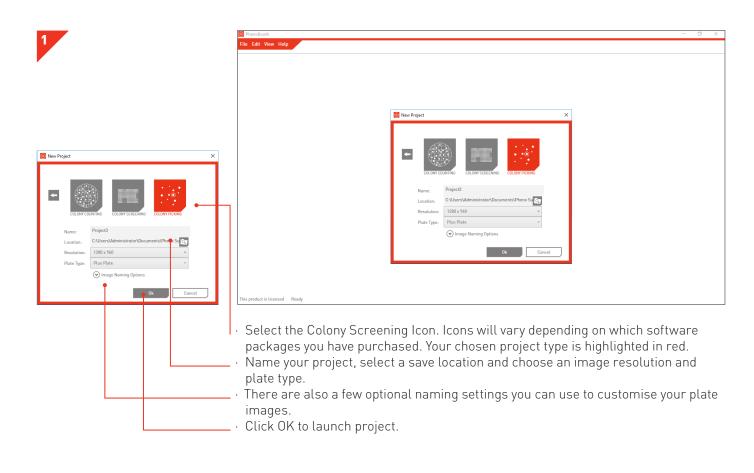
COLONY PICKING PROJECT

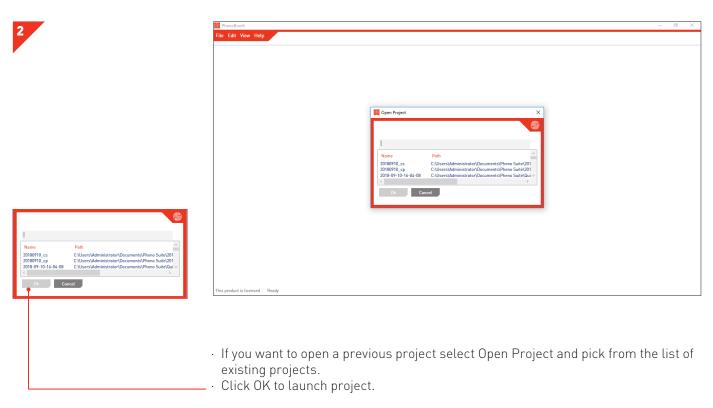
The Colony Picking package allows you to select individual colonies and export their coordinated to be picked using the Singer ROTOR. This section will guide you through the Colony Picking package for selection as well as the ROTOR software steps for picking.

There are 5 stages in the Colony Picking package: Image Acquisition, Pre-processing, Detection, Filtering and Stinger Export.

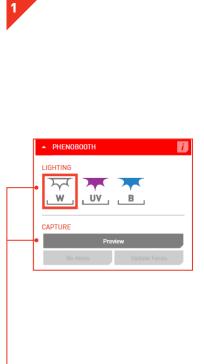


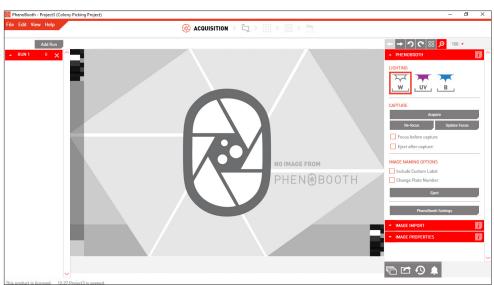
COLONY COUNTING - NEW PROJECT



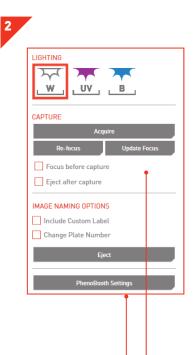


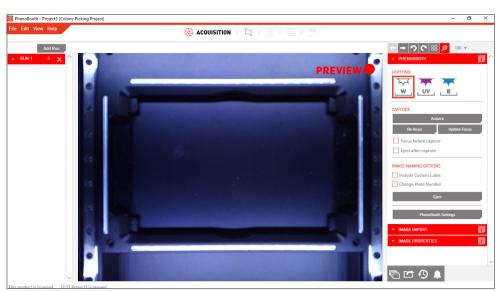
COLONY PICKING - IMAGE ACQUISITION





- There are two options for acquiring an image: PhenoBooth or import an existing image. However, using imported images for colony picking is not recommended because the plate may have changed since the photo was taken, compromising accuracy.
- · Select your lighting settings and press Preview to preview a live image from PhenoBooth.





- · If using the PhenoBooth, click on that tab and select your desired light settings. Plate names are automatically generated.
- · Insert a plate into PhenoBooth and press Acquire.
- · Eject will allow you to remove the plate.
- Repeated until all of your plates have been captured.
- moves onto the pre-processing stage.

COLONY PICKING - IMAGE ACQUISITION







- \cdot Your captured images now appear on the left of the screen. The currently selected plate is highlighted with red.

 → moves onto the pre-processing stage.

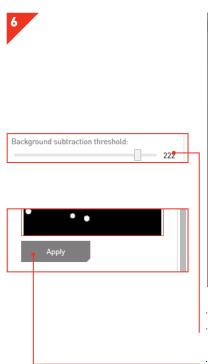
COLONY PICKING-PRE-PROCESSING & DETECTION







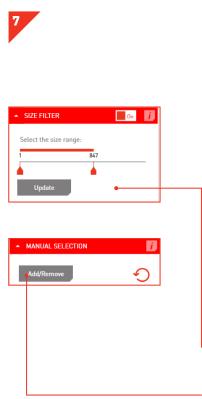
- · In the Crop plug-in select Guided Select.
- \cdot The grid will automatically add a crop preview to the plate guides. Press Crop to remove unnecessary background.
- moves to the Detection stage.





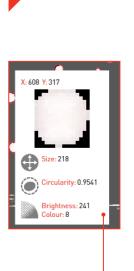
- · The Background Subtraction plug-in is used to remove the plate background.
- · Use the preview window to view colonies. Move the filter bar if necessary to adjust the filter intensity.
- · When you're happy with the preview image, press Apply to remove the background.
- . **•** moves to Filtering.

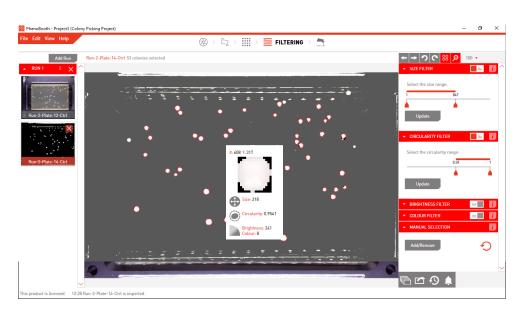
COLONY PICKING - FILTERING





- Detected colonies are highlighted with a red circle. 88 turns these highlights on or off.
- Use the filter options to set minimum and maximum values for size, circularity, brightness and colour for colony detection. You can use the size filter to discard items that are too large or small to be colonies. Click the Update button to apply.
- · Colonies can also be manually selected by clicking the Add/Remove button in the Manual Selection plug-in tab. Then simply click on a colony to select or deselect it.
- · You can batch process the rest of your plates with the same options using the instructions on p.47.

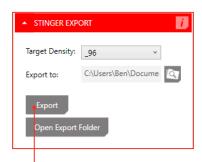


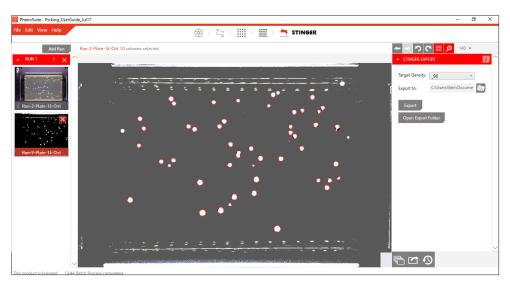


- · Hover over a colony to see it's size, circularity and colour properties in the Colony Info plug-in window.
- . moves onto the Stinger stage.

singerinstruments.com

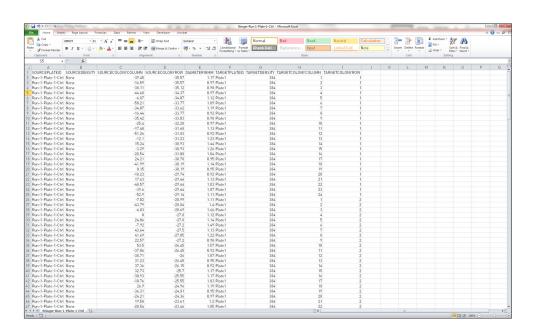




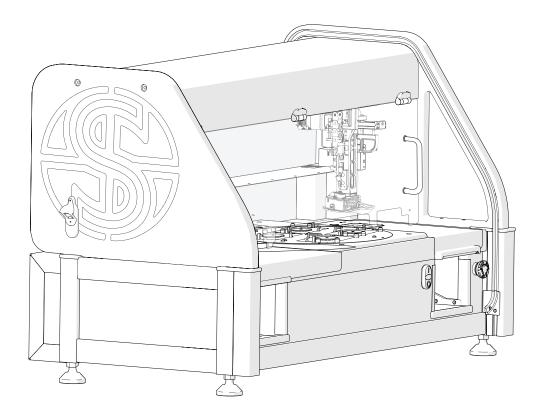


- · Use the Stinger plugin to export colony locations for picking.
- Select the density you want the colonies arrayed in (96, 383, 1536 or 6144), select the file location and then press Export.
- The file will need to be saved to a USB stick so it can be transferred to the ROTOR HDA.
- \cdot You can batch process the rest of your plates with the same options using the instructions on p.47.





- · The exported .CSV file contains all information on the selected colonies.
- · It's now time to load this file into the ROTOR HDA.

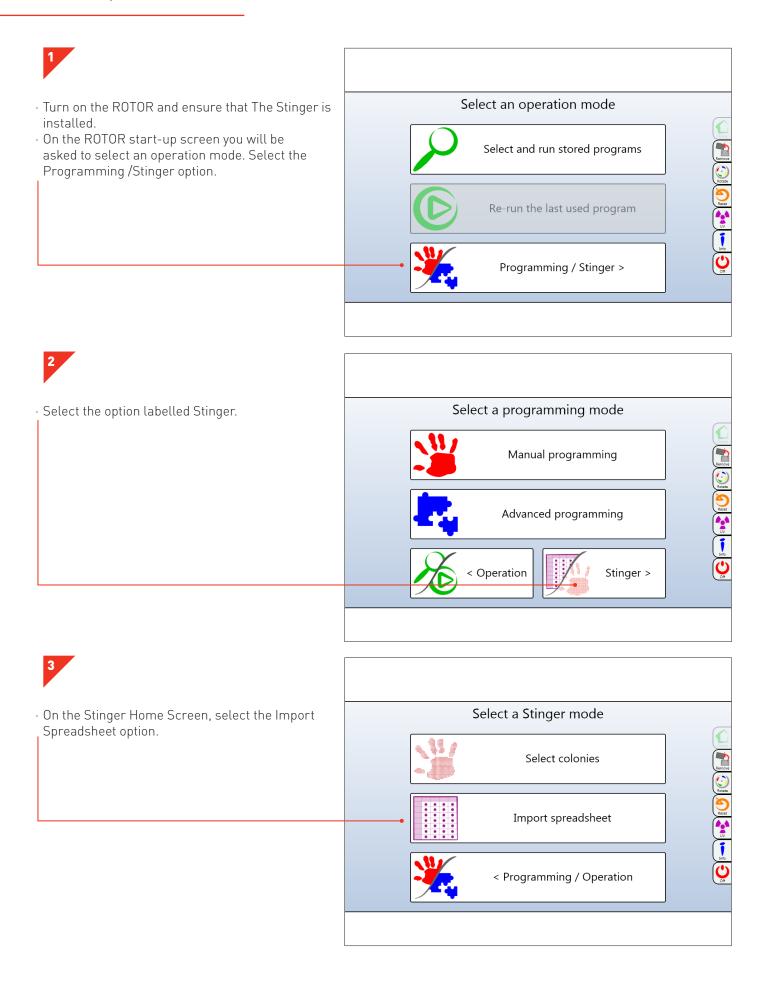


Now head over to the ROTOR HDA.

Please make sure The Stinger single colony picker is installed.

singerinstruments.com

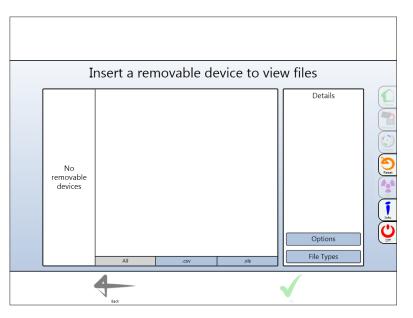
ROTOR HDA / THE STINGER

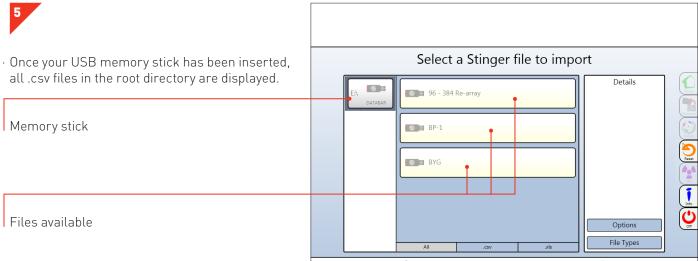


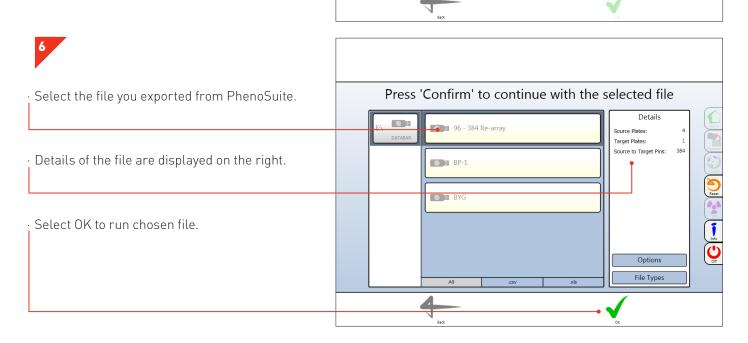
ROTOR HDA / THE STINGER



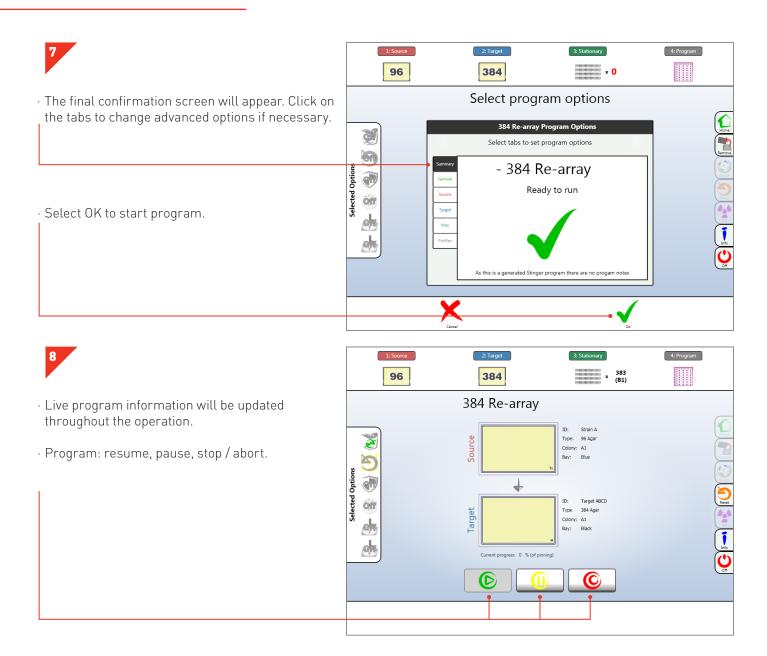
• This screen will appear if no USB memory stick is inserted. Insert your USB memory stick now.



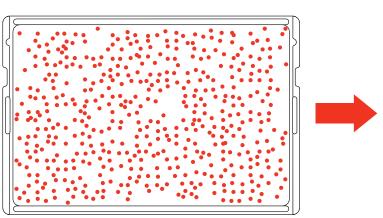




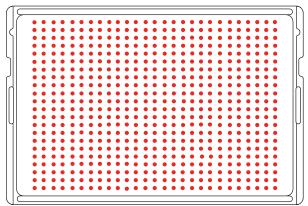
ROTOR HDA / THE STINGER



SOURCE PLATE



TARGET PLATE



BEST PRACTICE

We want you to have a trouble-free time with your PhenoBooth so we've assembled some handy hints, tips and troubleshooting answers to keep you on the straight and narrow.

singerinstruments.com

GENERAL TOOLS



PREVIOUS STAGE. If you want to change any settings in a previous stage, use this button to move to the previous stage.



UNDO. Undo the last process completed.



HIGHLIGHT COLONIES. Turn on or off detected colony highlights.



ZOOM PERCENTAGE. Selects zoom percentage of current plate.



NEXT STAGE. When you're happy with all the setting in the current stage, use this button to move to the next stage.



REDO. If you just used the undo tool, you can redo that process again with this button.



ZOOM. Use to zoom in on colonies of interest.

STAGE NAVIGATION



IMAGE ACQUISITION.



PRE-PROCESSING.



DETECTION.



FILTERING.



ANALYSIS. Colony Screening package only.



VISUALISATION. Colony Screening package only.



STINGER. Colony Picking package only.

SOFTWARE ICONS

NOTIFICATIONS



CONNECT PHENOBOOTH. PhenoBooth needs to be connected.



CONNECTION PROBLEM. There is a problem with the PhenoBooth connection. Try reconnecting the USB 3.0 cable less slowly, see p.2.



LIGHTING TIMED OUT. If left for too long the lighting goes on standby.



TRAY CRASH. The input tray has crashed. Ensure plate is loaded correctly and the lid has been removed.



CAPTURING. PhenoBooth is taking an image.



TRAY ACTIVE. Input tray is moving.

LIGHTING OPTIONS



WHITE TOP LIGHT. White, flat-field incident lighting.



UV TOP LIGHT. Ultra-violet, flat-field incident lighting.



BLUE TOP LIGHT. Blue, flat-field incident lighting.

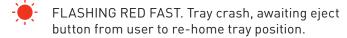


WHITE BOTTOM LIGHT. White, flat-field, transmitted light.

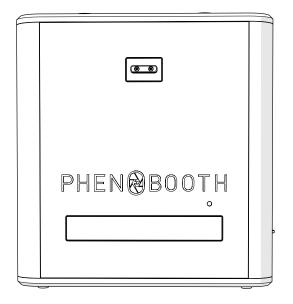
singerinstruments.com

FEEDBACK LIGHT

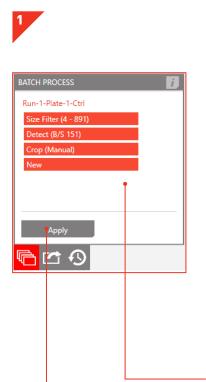


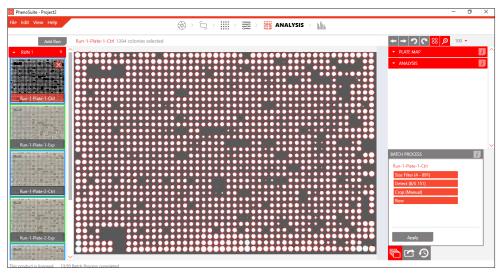






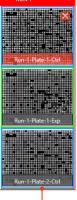
BATCH PROCESSING

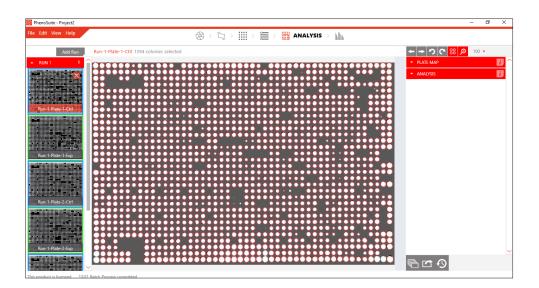




- Using the same settings for all plate images in a run will not only increase the speed of analysis but also ensure consistent and reliable information. A good time to batch process all of your plates is after all filtering and measuring has taken place.
- · Click the Batch Process tab and select which processes you want to apply to the rest of the images. The first click will select all processes.
- · Click Apply and batch processing will begin. This may take a while for larger numbers of images.



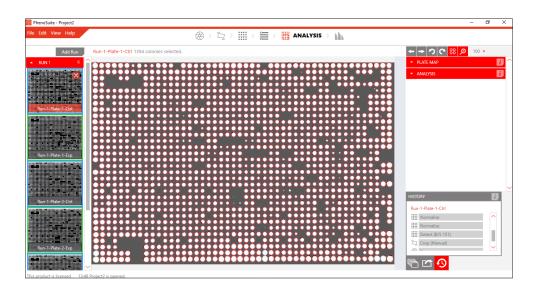




- · All plate images in your current run have now been processed with the same options as your original image.
- · You can see previews of the rest of your plates on the left of the screen. Click a plate to see a full screen view.



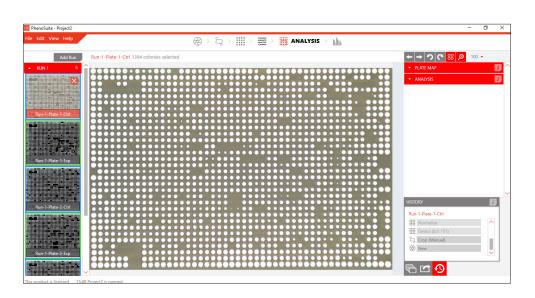




- · If you want to undo the last change you made, simply click the undo button: 🔈 .
- If you want to go back further then use the History plug-in. Open the History plug-in tab and select the process you want to revert back to. The list is in chronological order with the most recent process at the top.
- · In this example we're reverting back to when we cropped the image.



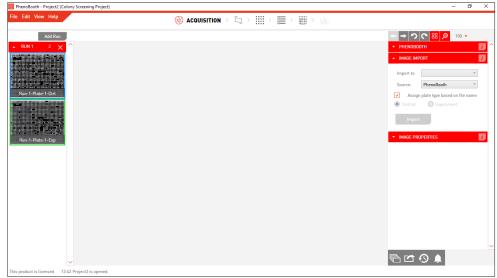




- · Now, the last process applied to the image is crop. All processes after crop have been discarded.
- Discarded processes are marked as light grey in the History plug-in. You can revisit any of these processes by clicking on them, until you make a change to the plate image outside of the History plug-in.

ADD NEW PLATE RUN

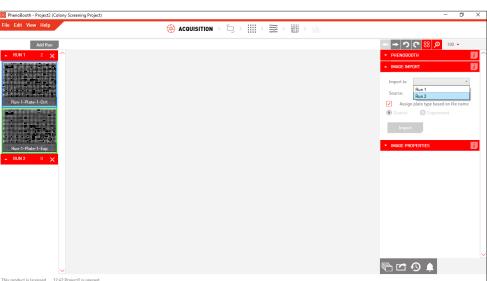






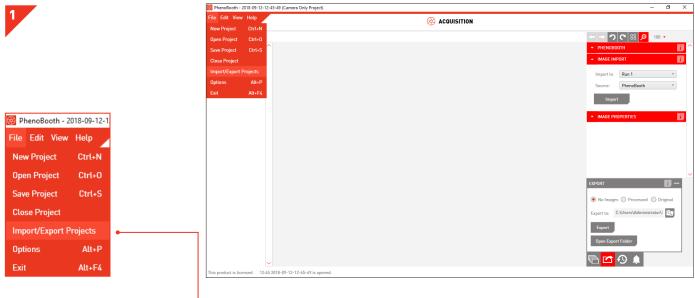
- · Your plates can be organised into separate runs for different groups of experiments.
- · To add a new run, simply click Add Run in the Plate Preview section on the left of the screen.



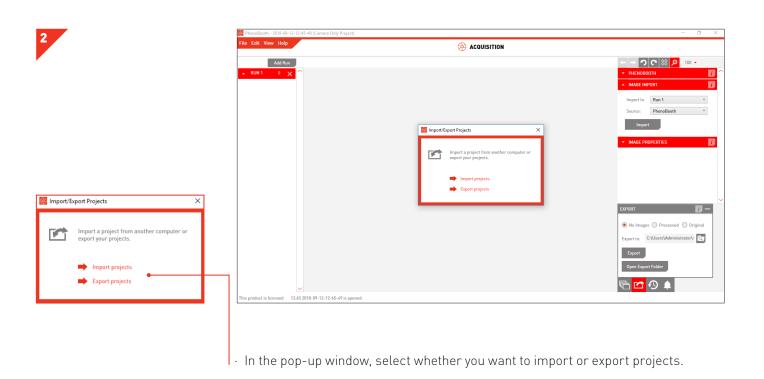


- · When acquiring images, whether capturing using a PhenoBooth or importing existing images, you'll be asked to select which run you want your images to be filed under.
- · Here you will see all the runs you have added so far.

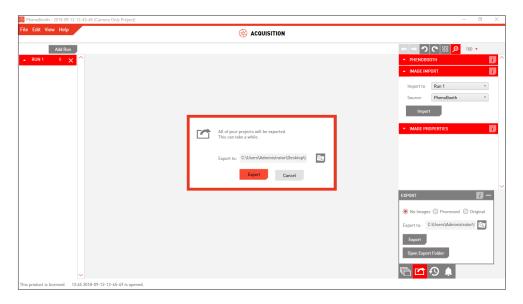
IMPORT / EXPORT PROJECTS



· PhenoBooth allows you to move projects from computer to computer. To do this, select the Import/Export Projects option in the File menu.

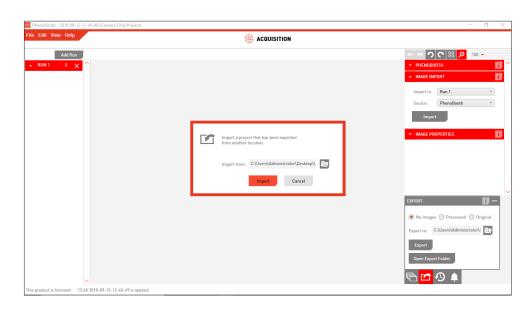






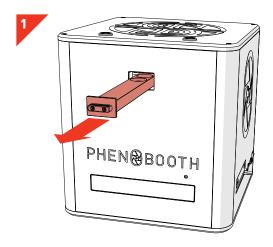
 \cdot For exporting, select the export location and then press Export.





· For importing, select the import location and then press Import.

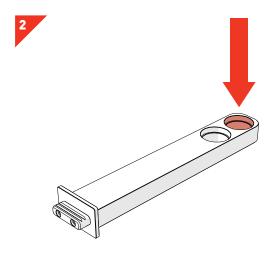
USING FILTERS



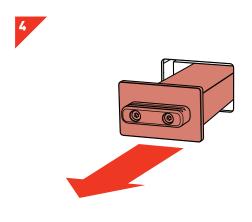
· Remove filter tray fully.



· Reinsert the filter tray fully until you feel the click. This will set the tray to the no filter position.



- · Place the desired filter into the end filter slot.
- The PhenoBooth accepts standard 25mm circular filters with a maximum height of 5mm.



 $\cdot\,$ To select the filter for use, pull the tray out slightly to the filter position — you will feel it click into place.

NOTES

singerinstruments.com



Roadwater, Watchet, Somerset. TA23 ORE UK

+44 (0)1984 640226 (tel) +44 (0)1984 641166 (fax)

contact@singerinstruments.com singerinstruments.com





SCAN TO VISIT WEBSITE FOR MORE HELPFUL TIPS AND TUTORIALS!

